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Author	Chun Ik Lim1,2, Hyo Jun Choo2, Jae Hong Park3
Affiliation	1 Department of Animal Science, Jeonbuk National University, Jeonju, 54896, Republic of Korea 2 Poultry Research Institute, National Institute of Animal Science, RDA, Pyeongchang, 25342, Republic of Korea 3 Department of Animal Resource & Science, Dankook National University, Cheonan, 31116, Republic of Korea
ORCID (for more information, please visit https://orcid.org)	Chun Ik Lim (https://orcid.org/0000-0003-0386-5694) Hyo Jun Choo (https://orcid.org/0000-0002-7747-5077) Jae Hong Park (https://orcid.org/0000-0002-2025-0141)
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CORRESPONDING AUTHOR CONTACT INFORMATION

For the corresponding author	Fill in information in each box below
First name, middle initial, last name	Jae-Hong Park
Email address – this is where your proofs will be sent	parkjh@dankook.ac.kr
Secondary Email address	atom1965@hanmail.net
Address	Department of Animal Resource & Science, Dankook National University, Cheonan, 31116, Korea
Cell phone number	+82-10-3853-9997
Office phone number	+82-41-550-3659
Fax number	+82-63-270-2612

Abstract

This study investigated the effect of dietary supplementation with phytase on growth performance, fecal excretion, and compost nutrition on broilers fed available phosphorus (avP)- and calcium (Ca)-deficient diets. A total of 750 one-day-old broiler chicks were randomly divided into five dietary groups having ten replications in a floor house. Diets of the groups were formulated with positive control (PC), negative control (NC; low avP and Ca), and NC supplemented with phytase levels; 500 (NC500), 1,000 (NC1000), and 1,500 FTU/kg (NC1500). A three-phase feeding program was used in the trial. Average daily gain (ADG) and average daily feed intake (ADFI) in the groups fed diets supplemented with phytase were significantly ($p < 0.05$) higher than those fed NC and the increase was equivalent to those fed PC. Serum levels of Ca and phosphorus (P) were higher ($p < 0.05$) in broilers fed NC1000 and NC1500 than in those fed NC. Interleukin (IL) level was the lowest in the group fed NC. Plasma *myo*-inositol (INS) concentrations in the NC1500 group were higher ($p < 0.05$) than PC, NC, and NC500 groups. Crude protein (CP) excretion was notably ($p < 0.05$) lower in the NC1500 group than in PC and NC groups. A lower ($p < 0.05$) concentration of P_2O_5 was observed in compost from the group fed NC1500 than the groups fed PC and NC. Accordingly, we suggest that phytase supplementation in lower avP and Ca levels of broiler diet can improve their productive performance and reduce environmental pollution.

Keywords: broiler, phytase, performance, fecal excretion, compost

Introduction

Poultry industry is growing globally. The increased productivity induces an increase in generated poultry excreta [1]. Manure compost from poultry is used as an organic fertilizer for soils low in nitrogen (N), due to its N, phosphorus (P), and potassium (K) levels. The global market for chicken manure has grown because of increased reliance on organic farming, agro-based industries, and poultry breeding [2]. However, excessive use of poultry-composts has caused adverse effects on air quality, water eutrophication, and soil acidification [3].

Poultry is unable to utilize P sufficiently from a grain-based diet due to their insufficient endogenous phytase production in the gastrointestinal tract. To achieve the P requirements of birds, inorganic phosphate supplementation is needed [4]. The inclusion of inorganic phosphate in their diet imposes a considerable cost; P is the third most expensive ingredient following energy and protein. Furthermore, anti-nutritional properties of phytate can cause the formation of an insoluble complex through mineral chelation and nutrient binding, as well as an increase in endogenous losses, which makes it harder for birds to digest and absorb nutrition, and negatively affect their performance [5]. The supplementation of exogenous phytase has been used in the poultry diet, especially lower level of P [4], calcium (Ca), and available P (avP) diet [6] to improve the productivity of broiler chickens. Another goal is to reduce the excretion of P and associated environmental pollution [7]. Improvement in the growth performance of broilers has been observed with phytase supplementation, which may increase the availability of nutrients by breaking down phytate, including inorganic phosphate, protein, and other minerals such as Ca, copper, iron, zinc, and sodium [8,9]. Furthermore, a good way to recycle organic P is composting animal feces. The compost from hens fed a diet supplemented with phytase (500 FTU/kg) is a major part of nutrient-balanced organic fertilizers [10]. The organic fertilizers can be used on crops without causing side effects [11]. Since poultry dietary management affects nutritional excretion, a research on the process among diet, feces, and compost could be needed to improve the usability of the poultry excretion in the fertilizer industry. To our knowledge, however most studies have confirmed the effect of phytase supplementation on nutritional excretion. there are no studies which have investigated nutritional values in composting using such excretions.

Therefore, this study aimed to evaluate the productive performance and excreted nutrients of broilers fed diets supplemented with various levels of phytase (500, 1,000, and 1,500 FTU/kg), and to confirm the nutritional value of mature compost from the excreted feces.

Materials and Methods

Birds and experimental diets

This study was carried out at the Poultry Experimental Station of the Department of Animal Sciences at Jeonbuk National University in the Republic of Korea. The protocols for the experiment were approved by the Jeonbuk National University Institutional Animal Care and Use Committee (JBNU 2021-0168).

A total of 750 one-day-old broiler chicks (Ross 308) were obtained from a local hatchery and randomly allocated to five dietary treatments in an environmentally controlled house (12.4 birds/m^2), with each treatment replicated ten times with 15 birds. Each pen was covered with rice husk as a bedding material on the floor. During the first seven days post-hatching the temperature was maintained in the room at 33℃, after which it was gradually reduced until each bird was 21 days old to 23±2℃, at which it was maintained throughout the remainder of the trial period. All diets were corn and soybean meal-based and were formulated in three phases; starter (0 to 11 d), grower (12 to 25 d), and finisher (26 to 42 d). The diets consisted of the positive control (PC) and the negative control (NC) in all phases. The PC was formulated to meet the nutrient requirements of the broilers as described in the Korean Feeding Standard for Poultry [12] while the NC was formulated with 0.15% less avP and 0.17% less Ca than the PC (Table 1). The NC was provided to three dietary groups with diets supplemented with phytase (Ronozyme HiPhos GT, DSM Nutritional Products) levels of 500 (NC500), 1,000 (NC1000), and 1,500 FTU/kg (NC1500). All experimental diets were provided in mash form throughout the trial period. Birds were offered free access to feed via round feeders and fresh drinking water was served via a nipple drinker system.

Growth performances

Birds were randomly weighed from chick boxes on arrival to obtain information as to their average weight, thereby ensuring there were no statistically significant differences in starting pen weight between the treatment groups. Average daily gain (ADG) and average daily feed intake (ADFI) were later measured over the experimental period (42 d). Feed conversion ratio (FCR) was calculated by dividing the ADFI by the ADG.

Blood profiles

Approximately 3 ml of blood was collected from the wing vein of each bird (ten birds per treatment) by a sterile syringe needle at the end of the experimental period. The serum was separated by centrifugation at 3,000 rpm at 4ºC for 15 minutes. Separated serum was put into Eppendorf tubes and stored at -20ºC until analysis. Serum Ca and P concentrations were measured by commercial diagnostic kits using an automatic blood biochemistry analyzer (Konelab 20 analyzer, Thermo Fisher Scientific, Vantaa, Finland). Serum interleukin (IL)-2 and IL-6 concentrations were analyzed using ELISA kits (Elabscience Biotechnology Co., Ltd., E-EL-Ch0120 and E-EL-Ch0228, respectively, Houston, TX, USA) according to the manufacturer's instructions. Absorbances were read at 450 nm using a microplate spectrophotometer (BioTek ELX 800, Winooski, VT, USA). Plasma *myo*-inositol (INS) concentration was analyzed using the method described by Pirgozliev et al. [13]. Collected blood samples from ten birds per treatment were immediately kept into heparinized. The samples were mixed with 2 ml of ice-cold 5% w/v perchloric acid and maintained on ice for 20 min to precipitate protein. The samples were then centrifuged at 16,000 × *g* for 15 min at 4°C, and the supernatant was diluted 50-fold in 18.2 MOhm.cm water. The samples (20 µL) were then injected into a 4 mm × 50 mm CarboPac PA1 column (Dionex, UK). INS was determined by high-performance liquid chromatography (HPLC) pulsed amperometry (HPLC-PAD) on a gold electrode at 30°C after separation by 2-dimensional HPLC (Dionex DX-600 HPLC System).

Fecal excretions

At the end of the experiment, a total of male thirty birds whose body weight was closest to the mean were selected, including six birds from each treatment group, and placed in an individual metabolic cage (the dimension of each cage was 0.35 m × 0.43 m) which was fastened for 24 hours. The digestive trial period

lasted for 7 d and included 4 d of acclimation to diet and environment and 3 d of excreta sample collection. Excreta samples were collected into plastic bags from each cage and immediately frozen at -20℃. After raw excreta (RE), dry excreta (DE), and moisture (MS) contents were measured, the samples were finely ground and passed through a one-millimeter sieve and then analyzed for N and P excretions per feed intake (kg) by AOAC [14]. CP concentration was calculated by multiplying 6.25 by the N concentration.

Composts

At the end of the analysis of experimental fecal excretion, in total, we collected the feces from thirty birds (six birds per group), each held in an individual cage for 10 d. Each fecal sample (5 kg) was matured for one month in an independent composting facility with heating (40℃) and ventilation (using the compost maturity method of Walker [15]) until turning blackish-brown without the form and smell of feces. Briefly, water was supplied so that the moisture content of the broiler feces could be maintained at about 60%. Additionally, to prevent the solidification of the feces and supply fresh air, the feces were stirred 3 times daily. After hydrolyzing the water sample, the produced compost was analyzed using the Kjeldahl method for N, Vanadate method for P_2O_5, and inductively coupled plasma atomic absorption spectrophotometer (ICP-OES, GBC Scientific Equipment Ltd., Australia) for K_2O. For pH analysis, the ratio of the samples to distilled water was 1:5 (wt. %). The mixture was stirred for 1 hour and filtered through a glass fiber filter paper (GF/C Filter), and the filtrate was measured with a pH meter (HANA Co, HI-2222, USA).

Statistical analysis

All data were analyzed using one-way analysis of variance (ANOVA) of SAS software (SAS 9.1, 2009, SAS Institute Inc., Cary NC, USA) in a completely randomized design. The means of different dietary groups were compared with Duncan multiple range tests. Significant differences were determined at $p < 0.05$.

Results

Productive performance

The productive performances of broilers fed experimental diets are presented in Table 2. The ADG of broilers fed NC500, NC1000, and NC1500 was significantly ($p < 0.05$) higher than those fed NC and the increase was equivalent to those fed PC. The higher ADFI was confirmed in birds fed NC1000, NC1500, and PC than in those fed NC. However, groups fed diets supplemented with phytase showed no changes FCR compared to groups fed PC and NC.

Blood profiles

In blood concentrations (Table 3), serum Ca level was increased ($p < 0.05$) in the NC1000- and NC1500-fed groups than in NC- and NC500-fed groups. In addition, the serum Ca level in broilers fed NC1500 was higher ($p < 0.05$) than those fed PC. A significant increase ($p < 0.05$) in serum P levels was observed in birds fed NC1000 and NC1500 compared to those fed NC. Both IL-2 and IL-6 levels were significantly ($p < 0.05$) lower in the group fed NC than in the other groups. Plasma INS concentration in broilers fed NC1500 was higher ($p < 0.05$) than those fed PC, NC, and NC500.

Fecal excretion

The fecal excretion per feed intake (1 kg) of broilers fed experimental diets is shown in Table 4. There were no significant differences in excretions of RE, DE, and MS among the dietary groups. However, the CP excretion of birds fed NC500, NC1000, and NC1500 was significantly ($p < 0.05$) lower than those fed PC diet. The excretion of P was significantly lower ($p < 0.05$) in broilers fed NC, NC1000, and NC1500 than in those fed PC.

Compost characteristics

Table 5 reflects that no differences were found regarding most compost characteristics (N, K_2O, MS, and pH). However, the P_2O_5 levels were significantly ($p < 0.05$) lower in the group fed NC1500 than in the groups fed PC and NC.

Discussion

Phytase supplementation to a diet low in avP and Ca is well-documented as improving the growth performance of broilers [4,6,16]. The breakdown of phytate complexes by phytase provides a more available source of P and other dietary essential nutrients, which in turn increase productive performance (ADG, ADFI, and FCR) in birds with diets low in avP [17] or low in both Ca and avP [6]. The growth-improving effect of the phytase-supplemented NC diet in our study (Table 2) may therefore be attributable to the fact that phytase, at all levels, facilitates the utilization of nutrients (Ca and avP).

The serum Ca and P concentrations are major indicators of poultry nutritional status of Ca and P. In broilers fed a low avP and Ca diet, the regulatory mechanism orchestrates to maintain normal Ca and P levels by taking these away from the bones [18]. In our study, dietary supplementation with phytase in broilers fed a low avP and Ca diet increased serum Ca and P concentrations (Table 3), indicating that phytase enhances the availability of Ca and P by hydrolyzing phytate-bound molecules. These findings are supported by Liu et al. [19], who reported that the serum concentrations of Ca and P were decreased in birds fed a diet low in Ca and P, and then increased with the dietary supplement of phytase (500 and 1,000 FTU/kg).

INS, which is the final product of degradation produced by the binding of phytase to phytate, plays an important role in maintaining phospholipid structures, lipid metabolism, cell signaling, and cell growth [20]. We confirmed that plasma INS concentrations were improved in birds fed NC1500 diets compared to those fed PC and NC diets (Table 3), suggesting that phytase supplementation degraded the phytates. Cowieson et al. [21] also reported significantly elevated levels of INS in the plasma of birds by the addition of phytase to their diets. Similarly, Summerfeld et al. [22] showed that phytase supplementation can increase the concentration of INS in the blood of broilers, and Beeson et al. [23] showed that dietary phytase can also increase it in their gizzard, ileum, and excreta.

The expression of IL proteins plays an important role in the differentiation and proliferation of immune cells, which in turn are important indicators of humoral immunity in chickens [24]. Th1 cells are known to excrete pro-inflammatory cytokines, such as TNF-α, IL-2 and IL-12, while Th2 cells secrete anti-inflammatory cytokines, such as IL-4, IL-5, IL-6 and IL-10 [25]. We showed in this study that the concentration of serum ILs (IL-2 and IL-6) increased in birds fed NC diets enhanced with any level of phytase, and the increase is statistically similar to that of the PC diet (Table 3). Khan et al. [26] suggested

that ILs enhanced some cell-mediated immune responses of broiler chickens by modulating macrophage activity in response to enzyme supplementation in a diet. We postulate that some of these effects are mediated by cytokines secreted from immune cells stimulated with the enzyme. To the best of our knowledge, no study has reported effects of phytase supplemtation as feed additives on interleukins of broilers. However, according to one relevant report [27], it demonstrated a higher serum cytokine activity in birds fed feed additives (direct-fed microbes and enzyme combination), suggesting that the nutritional improvements associated with enzymes may in part be mediated through immunocyte activity. Accordingly, we suggest that a diet supplemented with 500 to 1,500 FTU/kg phytase enhanced humoral immunity in chickens by releasing nutrients from phytate complexes and mitigating anti-nutritive properties of phytate.

Reducing the amount of CP and P in poultry manure is particularly important to lower the pollution of soil and water [28]. We confirmed that the CP and P excretions of broilers fed the NC1500 diet 22% and 32.6% lower, respectively than those fed the PC diet (Table 4). These results are supported by Srikanthithansan et al. [29] who observed that less P was excreted by broilers fed a low phosphorus diet (3.0% avP/kg) supplemented with 500 to 1000 FTU/kg phytase than those fed a normal P diet (4.5% avP/kg). Walk and Olukosi [30] also reported that broilers fed a phytase-supplemented diet (2000 or 4000 FTU/kg) showed a higher CP digestibility, together with less CP excreted.

The high level of P pollution in the poultry industry is a result of the intensity of poultry production; P inputs to diets and composts often exceed P outputs in crops. When the compost is applied to soil, the soil could contain excessive residual P. Since this would lead to soil and groundwater pollution, and disturb the entire ecosystem [28], the nutrition values of the compost from poultry feces should be considered for their application to soil. To our knowledge, only few studies have investigated the N, P, and K concentrations of compost matured from feces of the broilers supplemented with phytase. Nevertheless, according to one relevant report [31], dietary phytase supplementation (500 FTU/kg) improves ileal digestibility in broilers and significantly decreases N, P, and K concentrations in excreta. Our experiments likewise showed a decrease in P levels in the fecal excreta of the phytase-fed groups (Table 4). Furthermore, we confirmed a decrease in P_2O_5 levels in the compost from broilers fed a diet that included 1,500 FTU/kg phytase (Table 5). It is suggested that a compost based on the excreta of phytase-fed chickens reduces water-extractable P

runoff [11]. Although our finding showed less CP excretions of the NC 1500 group than those of the PC and NC groups ($p < 0.05$; Table 4), the N levels in the compost were not a significantly different among the group ($p > 0.05$; Table 5). The N contents in compost may be affected by the maturation process of compost caused in the huge variation of moisture content, N loss, and weight from feces of each group [32,33]. Since poultry compost which has rich N, P, and K concentrations is used for efficient crop growth, their NPK balance must be considered important in the soil [34,35]. The optimal NPK balance not only improves the growth of crops by providing nutrients that are lacking to crops, but also reduces environmental pollution by mitigating the excess of some nutrients in the soil [10,28,36]. In our findings, although the P content in compost from chickens fed dietary phytase is reduced, the compost may be used to maintain optimal NPK balance in soils with excess P.

Conclusion

The results from the current study showed the dietary supplementation of phytase in the broiler diet increase the productive performance, the nutritional digestibility and improve their immunity. Also, the phytase supplements to broiler could potentially could reduce environmental pollution through low CP and P excretions, as well as P_2O_5 levels in composts matured from the feces.

Acknowledgments

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1. References

1. Persistence Market Research (PMR). Chicken Manure Fertilizer Market - Global Industry Analysis and Opportunity Assessment 2021-2031. New York, USA. 2022. http://www.persistencemarketresearch.com/market-research/chicken-manure-fertilizer-market.asp

2. Ahn T, Kim D, Lee H, Shin H, Chung E. A study on the nutrient composition and heavy metal contents in livestock manure compost· liquefied fertilizer. J Korean Soc Water Environ. 2021;37:306-14. https://doi.org/10.15681/KSWE.2021.37.4.306

3. Zhang L, Li L, Pan X, Shi Z, Feng X, Gong B, Wang L. Enhanced growth and activities of the dominant functional microbiota of chicken manure composts in the presence of maize straw. Front Microbiol. 2018;9:1131-41. https://doi.org/10.3389/fmicb.2018.01131

4. Farhadi D, Karimi A, Sadeghi G, Rostamzadeh J, Bedford MR. Effects of a high dose of microbial phytase and myo-inositol supplementation on growth performance, tibia mineralization, nutrient digestibility, litter moisture content, and foot problems in broiler chickens fed phosphorus-deficient diets. Poult Sci. 2017;96:3664-75. https://doi.org/10.3382/ps/pex186

5. Woyengo TA, Nyachoti CM. Anti-nutritional effects of phytic acid in diets for pigs and poultry–current knowledge and directions for future research. Can J Anim Sci. 2013;93:9-21. https://doi.org/10.4141/cjas2012-017

6. Walk CL, Bedford MR, Santos TS, Paiva D, Bradley JR, Wladecki H, McElroy AP. Extra-phosphoric effects of superdoses of a novel microbial phytase. Poult Sci. 2013;92:719-25. https://doi.org/10.3382/ps.2012-02727

7. Angel R, Tamim NM, Applegate TJ, Dhandu AS, Ellestad LE. Phytic acid chemistry: influence on phytin-phosphorus availability and phytase efficacy. J Appl Poult Res. 2002;11:471-80. https://doi.org/10.1093/japr/11.4.471

8. Adeola O, Cowieson AJ. Board-invited review: opportunities and challenges in using exogenous enzymes to improve nonruminant animal production. J Anim Sci. 2011;89:3189-218. https://doi.org/10.2527/jas.2010-3715

9. Pirgozliev V, Bedford MR, Oduguwa O, Acamovic T, Allymehr M. The effect of supplementary bacterial phytase on dietary metabolisable energy, nutrient retention and endogenous losses in precision fed broiler chickens. J Anim Physiol Anim Nutr. 2012;96:52-7. https://doi.org/10.1111/j.1439-0396.2010.01121.x

10. Lim CI, Kim SJ, Kim JE, Song SE, Lee DB, Ryu KS. Study on Composition and Fertilization of Feces from Laying Hens Fed Dietary Phytase. Korean J Poult Sci. 2022;49:25-32. https://doi.org/10.5536/KJPS.2022.49.1.25

11. Vadas PA, Meisinger JJ, Sikora LJ, McMurtry JP, Sefton AE. Effect of poultry diet on phosphorus in runoff from soils amended with poultry manure and compost. J Environ Qual. 2004;33:1845-54. https://doi.org/10.2134/jeq2004.1845

12. Korean Feeding Standard for Poultry. Nutrient Requirement of Poultry. National Institute of Animal Science, RDA, Suwon, Korea. 2017.

13. Pirgozliev V, Brearley CA, Rose SP, Mansbridge SC. Manipulation of plasma myo-inositol in broiler chickens: effect on growth performance, dietary energy, nutrient availability, and hepatic function. Poult Sci. 2019;98:260-8. https://doi.org/10.3382/ps/pey341

14. Association of Official Analytical Chemists International (AOAC). Official Methods of Analysis. 16th ed. Association of Official Analytical Chemists International, Gaithersburg, MD. 2020.

15. Walker F. On-Farm Composting of Poultry Litter. Pages 1-9 In: Agricultural Extension Service. The University of Tennessee. Knoxvile, USA. 2015.

16. Manobhavan M, Elangovan AV, Sridhar M, Shet D, Ajith S, Pal DT, Gowda NKS. Effect of super dosing of phytase on growth performance, ileal digestibility and bone characteristics in broilers fed corn–soya-based diets. J Anim physiol Anim Nutr. 2016;100:93-100. https://doi.org/10.1111/jpn.12341

17. Pirgozliev V, Bedford MR, Acamovic T, Mares P, Allymehr M. The effects of supplementary bacterial phytase on dietary energy and total tract amino acid digestibility when fed to young chickens. Br Poult Sci. 2011;52:245-54. https://doi.org/10.1080/00071668.2011.560596

18. Li X, Zhang D, Bryden WL. Calcium and phosphorus metabolism and nutrition of poultry: are current diets formulated in excess?. Anim Prod Sci. 2017;57:2304-10. https://doi.org/10.1071/AN17389

19. Liu SY, Bold RM, Plumstead PW, Selle PH. Effects of 500 and 1000 FTU/kg phytase supplementation of maize-based diets with two tiers of nutrient specifications on performance of broiler chickens. Anim Feed Sci Technol. 2015;207:159-67. https://doi.org/10.1016/j.anifeedsci.2015.06.002

20. Gonzalez-Uarquin F, Rodehutscord M, Huber K. Myo-inositol: its metabolism and potential implications for poultry nutrition—a review. Poult Sci. 2020;99:893-905. https://doi.org/10.1016/j.psj.2019.10.014

21. Cowieson AJ, Aureli R, Guggenbuhl P, Fru-Nji F. Possible involvement of myo-inositol in the physiological response of broilers to high doses of microbial phytase. Anim Prod Sci. 2014;55:710-9. https://doi.org/10.1071/AN14044

22. Sommerfeld V, Künzel S, Schollenberger M, Kühn I, Rodehutscord M. Influence of phytase or myo-inositol supplements on performance and phytate degradation products in the crop, ileum, and blood of broiler chickens. Poult Sci. 2018;97:920-29. https://doi.org/10.3382/ps/pex390

23. Beeson LA, Walk CL, Bedford MR, Olukosi OA. Hydrolysis of phytate to its lower esters can influence the growth performance and nutrient utilization of broilers with regular or super doses of phytase. Poult Sci. 2017;96:2243-53. https://doi.org/10.3382/ps/pex012

24. Akdis M, Aab A, Altunbulakli C, Azkur K, Costa RA, Crameri R, Akdis CA. Interleukins (from

IL-1 to IL-38), interferons, transforming growth factor β, and TNF-α: Receptors, functions, and roles in diseases. J Allergy Clin Immunol. 2016;138:984-1010. https://doi.org/10.1016/j.jaci.2016.06.033

25. Romagnani S. Biology of human TH1 and TH2 cells. J Clin Immunol. 1995;15:121-29. https://doi.org/10.1007/BF01543103

26. Khan SH, Atif M, Mukhtar N, Rehman A, Fareed G. Effects of supplementation of multi-enzyme and multi-species probiotic on production performance, egg quality, cholesterol level and immune system in laying hens. J Appl Anim Res. 2011;39:386-98. https://doi.org/10.1080/09712119.2011.621538

27. Dersjant-Li Y, Gibbs K, Awati A, Klasing KC. The effects of enzymes and direct fed microbial combination on performance and immune response of broilers under a coccidia challenge. J App Anim Nutr. 2016;4:1-14. https://doi.org/10.1017/jan.2016.2

28. Abbasi F, Fakhur-un-Nisa T, Liu J, Luo X, Abbasi IHR. Low digestibility of phytate phosphorus, their impacts on the environment, and phytase opportunity in the poultry industry. Environ Sci Pollut Res. 2019;26:9469-79. https://doi.org/10.1007/s11356-018-4000-0

29. Srikanthithasan K, Macelline SP, Wickramasuriya SS, Tharangani H, Jayasena DD, Heo JM. Effects of adding phytase from aspergillus niger to a low phosphorus diet on growth performance, tibia characteristics, phosphorus excretion, and meat quality of broilers 35 days after hatching. J Poult Sci. 2020;57:28-36. https://doi.org/10.2141/jpsa.0180143

30. Walk CL, Olukosi OA. Influence of graded concentrations of phytase in high-phytate diets on growth performance, apparent ileal amino acid digestibility, and phytate concentration in broilers from hatch to 28 D post-hatch. Poult Sci. 2019;98:3884-93. https://doi.org/10.3382/ps/pez106

31. Ravindran V, Cowieson AJ, Selle PH. Influence of dietary electrolyte balance and microbial phytase on growth performance, nutrient utilization, and excreta quality of broiler chickens. Poult Sci. 2008;87:677-88. https://doi.org/10.3382/ps.2007-00247

32. Lee J, Choi D, Ok YS, Lee SR, Kwon EE. Enhancement of energy recovery from chicken manure by pyrolysis in carbon dioxide. J Clean Prod. 2017;164:146-52. https://doi.org/10.1016/j.jclepro.2017.06.217

33. Shan G, Li W, Gao Y, Tan W, Xi B. Additives for reducing nitrogen loss during composting: A review. J Clean Prod. 2021;307:127308. https://doi.org/10.1016/j.jclepro.2021.127308

34. Boateng SA, Zickermann J, Kornahrens M. Poultry manure effect on growth and yield of maize. West Afr J Appl Ecol. 2006;9:12-8. https://doi.org/10.4314/wajae.v9i1.45682

35. Peng X, Peng Y, Yue K, Deng Y. Different responses of terrestrial C, N, and P pools and C/N/P ratios to P, NP, and NPK addition: a meta-analysis. Water Air Soil Pollut. 2017;228:1-13. https://doi.org/10.1007/s11270-017-3383-8

347 36. Deng M, Li Q. Optimization of the NPK Ratio for Vegetative Growth in Aeschynanthus
348 longicaulis. Hortic Sci Technol. 2022;40:663-71. https://doi.org/10.7235/HORT.20220060

349

350 Table 1. Ingredients and nutritional composition of experimental basal diets

Ingredients (g/kg)	Starter (0-11 d)		Grower (12-25 d)		Finisher (26-42 d)	
	PC	NC	PC	NC	PC	NC
Maize	432	441	491	501	556	568
Soybean meal (49%)	378	385	340	347	291	297
Wheat bran	74.5	76.0	60.7	62.0	39.7	40.5
Wheat	54.4	55.5	48.0	49.0	47.5	48.5
Soybean oil	14.1	10.0	16.4	12.0	25.5	21.0
Corn gluten meal	6.86	-	6.86	-	6.86	-
Limestone	12.4	11.0	11.3	9.90	10.3	8.90
Monocalcium phosphate	16.2	9.20	14.1	7.10	12.1	5.10
Iodized Salt	2.94	3.00	2.94	3.00	2.94	3.00
Vit-Min Premix[1]	2.00	2.00	2.00	2.00	2.00	2.00
Lysine-HCl (99%)	2.06	1.90	2.06	1.90	2.06	1.90
L-Arginine (99%)	0.98	1.00	0.98	1.00	0.98	1.00
DL-Methionine (99%)	2.84	2.90	2.45	2.50	2.06	2.10
Threonine (99%)	0.98	1.00	0.98	1.00	0.98	1.00
Valine (96.5%)	0.49	0.50	0.49	0.50	0.49	0.50
Total	1000					
Calculated composition						
ME (kcal/kg)	3,050		3,100		3,200	
CP (%)	23.0		21.5		19.5	
Ca (%)	0.97	0.80	0.88	0.71	0.79	0.62
avP (%)	0.45	0.30	0.40	0.25	0.35	0.20
Methionine (%)	0.630		0.570		0.510	
Lysine (%)	1.46		1.36		1.22	
Analyzed composition						
CP (%)	23.2	23.1	21.3	21.5	19.8	19.6
Ca (%)	1.001	0.813	0.896	0.730	0.799	0.642
P (%)	0.853	0.711	0.780	0.639	0.699	0.557

[1] Contains per kg: Vit A: 12,000 IU; Vit D3, 5,000 IU; Vit K3: 3 mg, Vit B1: 2 mg, Vit B2: 6 mg, Vit B6: 4 mg, Vit B12: 25 mg, biotin: 0.2 mg, folic acid: 0.2 mg, niacin: 70 mg, pantothenic acid: 20 mg, Cu: 20 mg, Co: 0.5 mg, Fe: 50 mg, I: 1,300 mg, Mn: 120 mg, Se: 0.3 mg, Zn: 100 mg.
PC: positive control, NC: negative control, ME: metabolic energy, CP: crude protein, Ca: calcium, avP: available phosphorus, P: phosphorus.

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354 Table 2. Effects of phytase supplementation on the productive performance of broilers (n=10)

	PC	NC	NC500	NC1000	NC1500	SEM	*p* value
ADG (g/d)	60.2[a]	56.5[b]	60.1[a]	60.3[a]	61.6[a]	0.41	0.001
ADFI (g/d)	99.0[a]	93.0[b]	96.6[ab]	98.2[a]	98.9[a]	0.64	0.009
FCR	1.64	1.65	1.61	1.63	1.61	0.02	0.756

355 [a,b] means within the column with no common superscripts differ significantly ($p < 0.05$).
356 PC: positive control, NC: negative control, NC500: NC+phytase at 500 FTU/kg, NC1000: NC+phytase at
357 1,000 FTU/kg, NC1500: NC+phytase at 1,500 FTU/kg.
358 SEM: standard error of mean, ADG: average daily gain, ADFI: average daily feed intake, FCR: feed
359 conversion ratio.
360

361 Table 3. Effects of phytase supplementation on blood concentrations of Ca, P, IL, and INS in broilers (n=10)

	PC	NC	NC500	NC1000	NC1500	SEM	*p* value
Ca (mg/dL)	126^{bc}	117^{c}	121^{c}	134^{ab}	140^{a}	2.07	0.001
P (mg/dL)	220^{a}	188^{c}	195^{bc}	211^{ab}	204^{abc}	3.34	0.013
IL-2 (pg/mL)	162^{a}	123^{b}	162^{a}	157^{a}	159^{a}	4.10	0.007
IL-6 (pg/mL)	170^{a}	128^{b}	176^{a}	169^{a}	168^{a}	4.73	0.004
INS (mg/dL)	74.7^{b}	67.3^{b}	80.2^{b}	109.5^{ab}	122.6^{a}	6.86	0.032

362 ^{a,b,c} means within the column with no common superscripts differ significantly ($p < 0.05$).
363 PC: positive control, NC: negative control, NC500: NC+phytase at 500 FTU/kg, NC1000: NC+phytase at
364 1,000 FTU/kg, NC1500: NC+phytase at 1,500 FTU/kg.
365 SEM: standard error of mean, Ca: calcium, P: phosphorus, IL: interleukin, INS: *myo*-inositol.
366

367 Table 4. Effects of phytase supplementation on the fecal excretion of broilers (n=6)

	PC	NC	NC500	NC1000	NC1500	SEM	*p* value
RE (g)	983	977	962	949	968	16.5	0.978
DE (g)	185	185	182	177	170	2.87	0.390
MS (g)	798	792	780	773	798	16.5	0.987
CP (g)	68.6^{a}	62.3^{ab}	58.2^{bc}	58.0^{bc}	53.5^{c}	1.42	0.006
P (g)	3.01^{a}	2.21^{bc}	2.72^{ab}	2.39^{bc}	2.03^{c}	0.098	0.006

368 ^{a,b,c} means within the column with no common superscripts differ significantly ($p < 0.05$).
369 PC: positive control, NC: negative control, NC500: NC+phytase at 500 FTU/kg, NC1000: NC+phytase at
370 1,000 FTU/kg, NC1500: NC+phytase at 1,500 FTU/kg.
371 SEM: standard error of mean, RE: raw excreta, DE: dry excreta, MS: moisture, CP: crude protein, P:
372 phosphorus.
373

374 Table 5. Effects of phytase supplementation on the nutritional characteristics of compost from broiler feces

375 (n=6)

	PC	NC	NC500	NC1000	NC1500	SEM	*p* value
N (%)	2.92	3.13	2.82	2.81	2.54	0.127	0.721
P_2O_5 (%)	1.76[a]	1.51[b]	1.42[bc]	1.35[bc]	1.21[c]	0.052	0.005
K_2O (%)	1.38	1.45	1.47	1.31	1.32	0.047	0.781
MS (%)	59.2	57.5	60.0	59.0	59.3	1.317	0.986
pH	7.09	7.08	6.95	6.92	6.97	0.062	0.882

376 [a,b,c] means within the column with no common superscripts differ significantly ($p < 0.05$).

377 PC: positive control, NC: negative control, NC500: NC+phytase at 500 FTU/kg, NC1000: NC+phytase at

378 1,000 FTU/kg, NC1500: NC+phytase at 1,500 FTU/kg.

379 SEM: standard error of mean, N: nitrogen, P_2O_5: phosphorus pentoxide, K_2O: potassium oxide, MS: moisture.

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